#34

Ex-1

SECTION
XIII
Drugs Used for Immunosuppression

CHAPTER
53 IMMUNOSUPPRESSIVE AGENTS

Robert E. Handschumacher

INTRODUCTION

Targets for the Actions of Immunosuppressive Drugs. Modification of immune function by pharmacological agents is emerging as a major area of therapeutics. The primary objective in the past has been to suppress the immune system to permit allotransplantation. However, the recent elucidation of the role of interleukins and related cytokines in a variety of other pathophysiological states has suggested new applications for agents that affect the production or action of these mediators.

A summary of the principal elements in the cellular and molecular cascades that are responsible for activation of the immune response is depicted in Figure 53–1 (for a detailed review, *see* Paul, 1989). Each element may be considered a potential site for pharmacological intervention. Activation of the immune system by "non-self" antigens (alloantigens) or "self" antigens (autoantigens) is generally believed to require processing of the antigen by phagocytic cells such as macrophages, monocytes, or related cells. The processing and subsequent presentation of the antigen to one of the subsets of lymphocytes is associated with the elaboration and secretion of a number of small proteins by the phagocytes. Prominent among these is interleukin 1 (IL-1). This initial series of reactions is blocked by the adrenocorticosteroids (*see* Chapter 60).

Activated phagocytic cells then communicate with thymus-derived lymphocytes (T cells), particularly helper T cells. Receptor sites on T cells are complex, in that they detect both the processed antigen in question and major histocompatibility complex (MHC) proteins on the antigen-presenting cell (*see* Perlmutter, 1989). A complex of at least five other transmembrane proteins, known collectively as the *cell differentiation complex 3* (CD3), is associated with the T-cell receptor. The role of this complex is incompletely understood, but it is essential to the activation process. The monoclonal antibody OKT3 is specific for epitopes in the CD3 complex, and it affords a means of inhibiting the functional interaction between antigen-presenting cells and T cells. T cells are commonly divided into two major subsets on the basis of their expression of either CD4, which recognizes MHC II–associated antigens (helper cells), or CD8, which recognizes MHC I–associated antigens (suppressor–cytolytic cells). The phenotypic characterization of these subsets of T cells is even more complex as a result of their expression of a variety of other surface proteins.

In response to these concerted stimuli, T

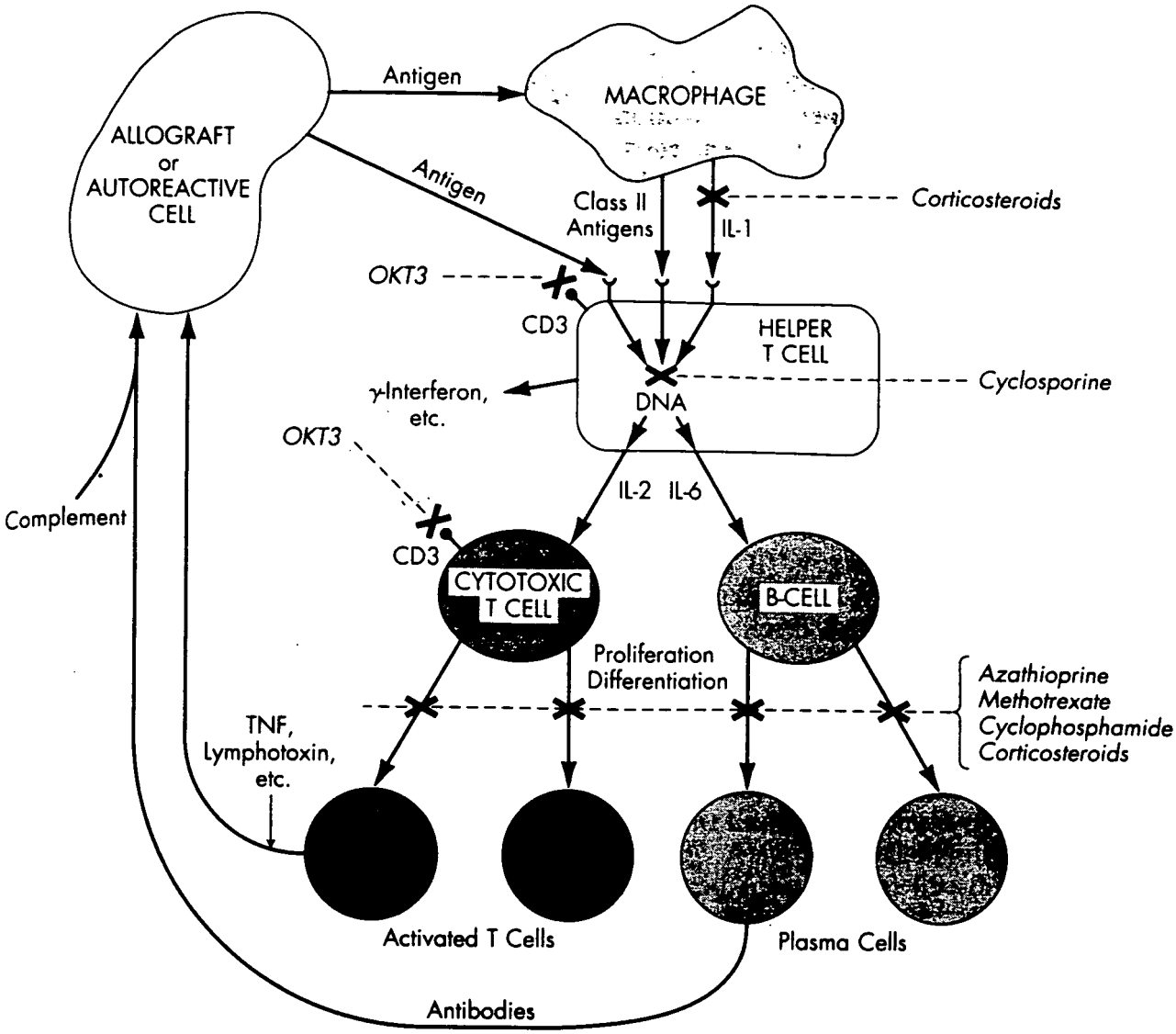


Figure 53–1. *Potential targets for immunosuppressive agents.*

The figure depicts the salient features of cellular and humoral immune responses and indicates the apparent sites of action of various immunosuppressive agents (*see* text for details). Abbreviations are: IL, interleukin; CD3, cell differentiation complex 3; OKT3, murine monoclonal antibody directed against an epitope in CD3; and TNF, tumor necrosis factor.

cells undergo clonal expansion, a proliferation process that requires both the expression of receptors for a growth factor, interleukin 2 (IL-2), and the production of IL-2 by T cells. Classical cytotoxic immunosuppressants such as methotrexate, azathioprine, and cyclophosphamide act by inhibiting the synthesis of DNA, thereby thwarting the stimulus for proliferation. The role of IL-1 in the activation of mature T cells is not clearly established, but it is an essential factor in the proliferation and differentiation of certain stem cells in the thymus that result in the emergence of mature T cells. Another consequence of the activation of helper T cells is the synthesis and release of a variety of cytokines that control both the cellular and humoral arms of the immune response (*see* Hamblin, 1988; Mizel, 1989; O'Garra *et al.*, 1988). It is this step in the activation of T cells that is exquisitely sensitive to cyclosporine and FK-506.

Cellular immunity is expressed as cytotoxicity toward target cells by activation of cytotoxic or "killer" T cells. Cytotoxicity is mediated both by direct cell–cell interactions and by secreted peptides, such as lymphotoxin, interferon, and tumor necrosis factor. The actions of the cytotoxic T

cells (which are facilitated by accessory cells) are also inhibited by adrenocorticosteroids. Another subpopulation of T cells can balance the antigen-specific activation process; these are termed suppressor cells. Their function results in regulation of cellular responses to antigens and inhibition of the production of antibodies via the humoral arm of the immune response. Stimulation of suppressor activity represents an additional means of achieving immunosuppression, but it has not yet been identified as an important aspect of the action of available immunosuppressant drugs.

The humoral arm of the immune response is responsible for the production of antibodies; this is carried out by cells derived from the bone marrow (B cells). In response to a battery of lymphokines that are primarily elaborated by T cells (notably IL-4, IL-5, and IL-6), antigen-specific B cells undergo clonal expansion and differentiate into a population of plasma cells—the primary source of circulating antibodies. This clonal expansion is also inhibited by the cytotoxic agents that inhibit the synthesis or function of DNA or RNA. A fraction of the B-cell population becomes "memory" cells, which can subsequently respond to the specific antigen more rapidly and with greater production of specific immunoglobulins.

Since certain autoimmune diseases, such as rheumatoid arthritis, nephrosis, uveitis, thyroiditis, and early stages of insulin-dependent diabetes mellitus, appear to involve responses to autoantigens, a potential role for immunosuppressive drugs has been recognized. Similarly, psoriasis and inflammatory bowel disease may reflect activation of cellular elements by some of the lymphokines that are essential for immune function (Gottlieb, 1988). For example, IL-1 is a potent endogenous pyrogen and is a growth factor for endothelial cells, synovial cells, and fibroblasts. IL-3 and IL-4 serve as growth factors for mast cells and as co-mitogens for hematopoietic cells (Mizel, 1989). Excess production of these substances as a consequence of inappropriate activation of immune cells can cause secondary effects by the release of a spectrum of inflammatory mediators (*e.g.*, histamine, eicosanoids, kinins). The agents discussed in this chapter are thought to ameliorate autoimmune and inflammatory states by reducing the elaboration and/or function of these trophic molecules at an early stage in the immune response (*see* Bach, 1989b).

Principal Uses and Limitations of Immunosuppressive Agents. Successful allograft transplantation has been possible for almost 30 years because of the availability of cytotoxic immunosuppressive agents. However, the introduction of cyclosporine and its use in combination with older immunosuppressants has made organ transplantation a more successful procedure that extends life for tens of thousands of patients every year (Bennett and Norman, 1986). Although renal transplants predominate, the frequency and success of cardiac and hepatic transplantations are increasing (Metzger and Hoffman, 1988; O'Grady *et al.*, 1988). The transplantation of other organs has been less successful, but progressive improvement has been evident. Several immunosuppressant drugs can also be used to manage a wide variety of autoimmune or inflammatory diseases. In some instances, these drugs are among the approved methods for treatment; these include azathioprine and methotrexate for rheumatoid arthritis, cyclophosphamide for nephrotic conditions of immune origin, and methotrexate for psoriasis. In other instances, clinical improvement has been demonstrated in experimental protocols. Examples include the beneficial effects of azathioprine in the treatment of nephrotic conditions, antibody-mediated thrombocytopenia, and autoimmune hemolytic anemia and of cyclosporine in patients with rheumatoid arthritis, uveitis, early-onset insulin-dependent diabetes mellitus, psoriasis, nephrotic syndrome, and aplastic anemia.

Modification of the immune response by pharmacological agents is most effective if therapy is begun before exposure to the antigen has an opportunity to generate a primary response (*e.g.*, pretreatment of allograft recipients). The secondary or anamnestic response is much less sensitive to the classical cytotoxic agents, but it can be suppressed with large doses of corticosteroids.

The latter agents are thus an important element in the treatment of acute graft rejection.

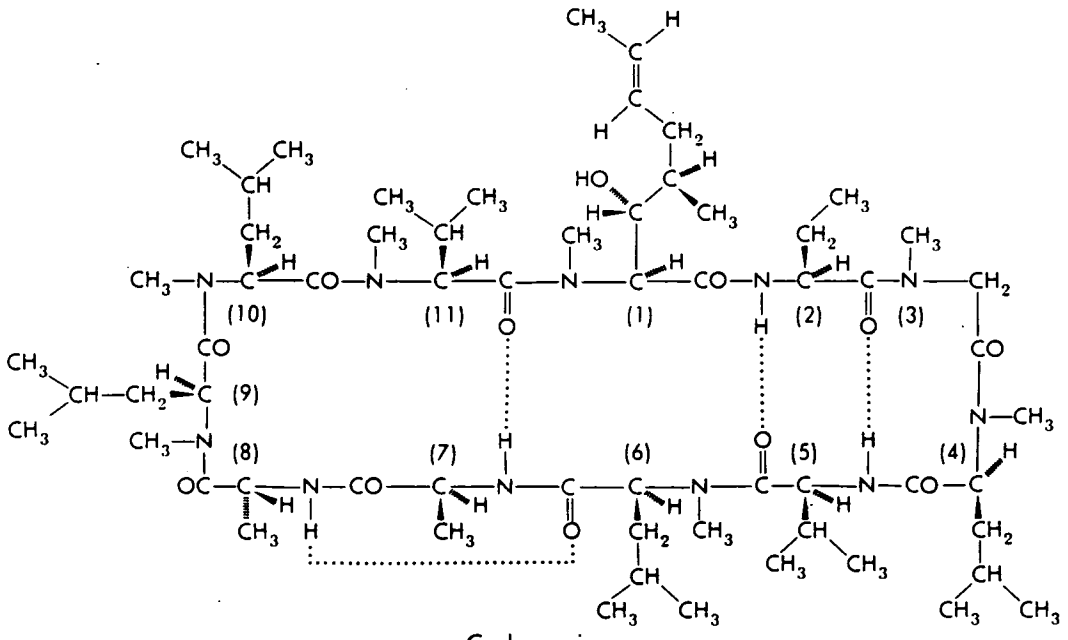
Fortunately, the sensitivity of lymphoid elements to the classical cytotoxic agents is somewhat greater than is that of other stem cells in the marrow, at least during the initial step of antigen recognition and during establishment of memory cells. However, the therapeutic index is narrow. When cytotoxic drugs are given for cancer chemotherapy, they are often given in large doses intermittently; this strategy is designed to permit lymphoid and other cellular elements to recover between courses. These drugs are given more continuously but in lower doses to produce immunosuppression.

Despite recent therapeutic advances, nonspecific suppression of the immune response to either autoantigens or alloantigens engenders an increased risk of infection by viral, bacterial, and fungal organisms (Bach, 1989a; Boitard and Bach, 1989). The same opportunistic pathogens that afflict patients with acquired immunodeficiency syndrome frequently limit therapy with immunosuppressive agents. The profound depression of immune surveillance by all of these drugs also greatly increases the incidence (3- to 100-fold) of malignant neoplasms in patients after allograft transplantation (Penn, 1986; Boitard and Bach, 1989). However, the use of these agents in patients with autoimmune disease has been associated with only a minimal increase in the incidence of cancer, perhaps because lower doses and shorter periods of treatment have usually been employed. Lymphomas and related neoplasms comprise a much larger fraction of all cancers in transplant patients than in the general population. This may result from stimulation of feedback mechanisms and hyperproliferation of stem cells that serve to restore immune function.

CYCLOSPORINE

Chemistry. Cyclosporine is one of a family of cyclic peptides produced by the fungus *Tolypocladium inflatum Gams;* it is comprised of 11 amino acid residues (*see* below). Cyclosporine is a very hydrophobic molecule with a unique 9-carbon amino acid in position 1 and a remarkable lack of other functional groups (Wenger, 1986). All amide nitrogens are either hydrogen bonded or methylated, and biological activity is very sensitive to alterations in stereochemical configuration and to modification of the residues at positions 1, 2, 3, 10, and 11. Cyclosporine contains a single D-amino acid residue in position 8, and the methyl amide between residues 9 and 10 is in the *cis* configuration; all other methyl amide moieties are in the *trans* form.

Pharmacological Effects and Mechanism of Action. The introduction of cyclosporine has provided an entirely new ap-



Cyclosporine

proach to immunosuppression by virtue of its highly selective ability to inhibit activation of T cells (Borel *et al.*, 1976; Borel, 1983; Shevach, 1985; Kahan and Bach, 1988). Unlike cytotoxic immunosuppressants, therapeutic concentrations of cyclosporine do not cause myelosuppression. Although its site of action has not been defined precisely, cyclosporine inhibits an early cellular response to antigenic and regulatory stimuli, primarily in populations of helper T cells (Kay and Benzie, 1984). Blockade of the pathways that effect the responsiveness of lymphocytes results in a spectrum of secondary changes in cellular function that generate both the therapeutic and unwanted effects of the drug (Drugge and Handschumacher, 1988).

The molecular targets for cyclosporine appear to be a family of proteins called *cyclophilins* (Handschumacher *et al.*, 1984; Harding *et al.*, 1986). These small proteins selectively bind cyclosporine and its active analogs with high affinity. Cyclophilins are abundant in lymphoid tissue, but isoforms of the protein are also found in varying proportions in most mammalian tissues (Koletsky *et al.*, 1986). Proteins with structures that are highly homologous to cyclosporine are present in essentially all eukaryotic organisms. The major isoform of cyclophilin has recently been shown to be identical to peptidyl proline *cis–trans* isomerase from porcine kidney (Fischer *et al.*, 1989; Takahashi *et al.*, 1989). This enzyme participates in the folding of proteins, presumably by assisting in the establishment of specific types of turns as nascent proteins assume their functional conformation. Thus far, all isoforms of this enzyme are inhibited by cyclosporine and its active analogs at concentrations that correlate with those required for their immunosuppressive effects. Studies with a large number of cyclosporine analogs indicate that either binding to cyclophilin or inhibition of isomerase activity is necessary but is not sufficient to ensure immunological activity *in vivo* (Quesniaux *et al.*, 1988).

Cyclosporine also binds with lower affinity to calmodulin; however, the relative affinity of cyclosporine analogs for calmodulin does not correlate with their immunosuppressive activity (Colombani *et al.*, 1985; LeGrue *et al.*, 1986; Foxwell *et al.*, 1988b). Nevertheless, this interaction with calmodulin may be responsible for some of the side effects of cyclosporine (*e.g.*, inhibition of protein phosphorylation) when plasma concentrations of the drug are elevated (Gschwendt *et al.*, 1987).

A direct connection has not yet been made between these molecular targets and the rapid and profound inhibition by cyclosporine of the production of IL-2 by helper T cells (Elliot *et al.*, 1984; Kronke *et al.*, 1984). Cyclosporine also causes a general reduction in the production and release of other lymphokines in response to an antigenic stimulus. At higher concentrations, cyclosporine inhibits expression of receptors for IL-2 (Herold *et al.*, 1986). Although cyclosporine can inhibit the activation of helper T cells, it does not prevent the stimulation of their clonal expansion by IL-2. It is potentially significant that cyclosporine allows the expression of suppressor cell activity at concentrations that inhibit the induction of cytotoxic T cells (Hess and Tutschka, 1980).

Pleotropic cellular responses to the administration of cyclosporine are well documented (Kahan and Bach, 1988). These include increased secretion of prolactin from the pituitary and reduced binding of prolactin to receptors on lymphocytes (Russell *et al.*, 1985; Larson, 1986). Patients treated with cyclosporine often have significantly increased concentrations of prolactin in the circulation. Variable effects on eicosanoid metabolism and Ca^{2+} fluxes have also been observed (Metcalf, 1984; Gelfand *et al.*, 1987). The relationship of these effects to cyclosporine-sensitive signal transduction mechanisms or to the role of cyclophilin is not at all clear.

Cyclosporine and its active analogs have other properties that seem quite unrelated to their actions on T cells and that may reflect in part the ubiquitous distribution of cyclophilins. A variety of parasitic infections including schistosomiasis and malaria respond to these compounds, presumably by a direct action on the parasite (Bueding *et al.*, 1981; Nickell *et al.*, 1982). Cyclosporine can also restore the sensitivity of cell lines and experimental tumors that are resistant to several cancer chemotherapeutic agents because of overexpression of the P-glycoprotein (the product of the MDR [multidrug-resistance] gene) (Slater *et al.*, 1986; Twentyman, 1988; Hait *et al.*, 1989). The mechanism of this effect is presumably unrelated to that responsible for immunosuppression, since both active and inactive analogs of cyclosporine can cause this effect.

Absorption, Fate, and Excretion. The oral bioavailability of cyclosporine varies from 20 to 50%; peak concentrations in plasma are achieved within 3 to 4 hours. About 60 to 70% of the drug in whole blood is contained in erythrocytes. Despite their small contribution to blood volume, leukocytes contain 10 to 20% of circulating cyclosporine. This concentration in leukocytes apparently reflects their content of cyclophilin; binding becomes saturated at plasma concentrations of cyclosporine in excess of 100 ng/ml (Foxwell, 1988a). The remainder of the drug circulates largely in association with plasma lipoproteins. Cyclosporine is also sequestered in tissues, and its apparent volume of distribution is rather large. It is cleared from blood with a half-life of about 6 hours, although wide variations in all pharmacokinetic parameters have been observed (Vine and Bowers, 1988; Kahan and Grevel, 1988).

Very little cyclosporine, or its metabolites, appears in the urine; most of the drug is excreted in the bile after metabolism in the liver (Maurer, 1985; Burckart *et al.*, 1986; Maurer and LeMaire, 1986). The cyclic peptide structure of cyclosporine is relatively resistant to attack, but cytochrome P_{450}–mediated oxidation of side chains is extensive (Aoyama *et al.*, 1989); hepatic dysfunction or concomitant administration of agents that affect the activity of cytochrome P_{450} causes dramatic changes in the elimination of cyclosporine (McMillan, 1989). Some of the metabolites have immunosuppressive activity, but their role in the therapeutic or toxic effects of the drug remains to be established (Freed *et al.*, 1987).

Drug Interactions. There have been many descriptions of effects of other drugs on the disposition of cyclosporine, but only a few of these interactions appear to be clinically significant. These reports are often difficult to evaluate because the methods used most commonly to analyze cyclosporine in whole blood detect both active and inactive metabolites (Vine and Bowers, 1988; McMillan, 1989). Accelerated clearance of cyclosporine has been demonstrated in patients receiving phenytoin, phenobarbital, trimethoprim–sulfamethoxazole, and rifampin, presumably as a result of induction of hepatic P_{450} systems. Administration of these drugs for the treatment of infections, seizures, or tuberculosis has caused rejection of transplanted organs because of reduced concentrations of cyclosporine in blood (McMillan, 1989). Decreased clearance of cyclosporine has been associated with concurrent administration of erythromycin, ketoconazole, or amphotericin B; this engenders a higher risk of toxicity from cyclosporine if its concentration in blood is not carefully monitored.

Clinical Toxicity. The major toxic manifestations of cyclosporine are renal, and nephrotoxicity occurs in 25 to 75% of patients treated with the drug. Although dose-related and usually reversible, nephrotoxicity frequently mandates cessation or modification of therapy (Mihatsch *et al.*, 1988; Racusen and Solez, 1988). The ultimate consequence is a reduction in glomerular filtration rate and renal plasma flow, but there is evidence of early damage to proximal tubules and to the endothelial and smooth muscle cells of small blood vessels. Plasma concentrations of creatinine and urea are used to guide dosage, but incipient rejection of a transplanted kidney will cause similar changes. Hypertension (10 to 15% elevation in blood pressure) is seen in more than 30% of patients with renal, hepatic, or cardiac transplants who receive cyclosporine (Bennett and Porter, 1988). Neurological toxicity is also common, especially in recipients of hepatic transplants (Walker and Brochstein, 1988); tremor occurs in over 50% of such patients and seizures in 5%. About 50% of patients who receive cyclosporine have elevated hepatic transaminase activities or concentrations of bilirubin in plasma; these abnormalities generally disappear if dosage is reduced or the drug is discontinued (Lorber *et al.*, 1987).

Treatment with cyclosporine is associated with an increased incidence of infections, but this problem is generally less prominent than with other immunosuppressant drugs (Kim and Perfect, 1989). There is a relatively low incidence of malignancies in patients who are treated with cyclosporine alone; however, when used in com-

bination with other agents, the drug causes malignant lymphomas with an unusually high incidence of brain metastases (Boitard and Bach, 1989).

Hirsutism and gingival hyperplasia are seen in 10 to 30% of patients who receive cyclosporine, but these reactions rarely affect therapy. Headache, paresthesias, flushing, sinusitis, gynecomastia, conjunctivitis, and tinnitus are observed occasionally. Although the drug is embryotoxic in animals, and its use should be avoided in pregnant women, many successful childbirths have occurred during therapy with regimens that include cyclosporine.

Therapeutic Uses. Cyclosporine is used primarily in combination with prednisone to sustain renal, hepatic, and cardiac transplants (Fowler and Schroeder, 1986; Kahan *et al.*, 1987; O'Grady *et al.*, 1988). The 1-year survival rate of grafts with cadaveric kidneys ranges from 70 to 85%; more than 60% of hepatic grafts currently function for at least 1 year. The 1-year survival rate after cardiac transplantation is higher than 80% in some centers. Success in pancreatic transplantation has improved significantly (Sutherland *et al.*, 1989); experience with the small bowel is limited.

Transplantation of allogeneic bone marrow has become a preferred treatment for many patients with aplastic anemia, acute nonlymphocytic leukemia, and severe combined immunodeficiency syndrome. Cyclosporine is used as an alternative to methotrexate to prevent the evolution of graft-versus-host disease in these patients; some protocols employ both agents (Storb *et al.*, 1989). Clinical trials indicate that cyclosporine may be useful in the treatment of a variety of autoimmune and related disorders, including rheumatoid arthritis (Shand and Richardson, 1988), glomerulonephritis, red cell aplasias (Totterman *et al.*, 1989), uveitis (Nussenblatt *et al.*, 1985), inflammatory bowel disease (Brynskov *et al.*, 1989; Sachar, 1989), and psoriasis (Bos, 1988; Van Joost *et al.*, 1988; von Graffenried, 1989). Rapid and dramatic results are seen in many patients at doses of cyclosporine (3 to 5 mg/kg) that rarely cause serious toxicity. However, disease relapses in a significant proportion of patients when therapy is terminated. Thus, cyclosporine cannot be considered curative, but may be most useful for acute exacerbations of these diseases when they have become refractory to conventional agents. In insulin-dependent diabetes mellitus, the administration of cyclosporine within the first 6 weeks of onset can reverse the condition temporarily, presumably by inhibition of an autoimmune reaction (Feutren *et al.*, 1986; Bach *et al.*, 1988). However, the prospects for long-term therapy are not encouraging.

Preparations and Dosage. *Cyclosporine* (SANDIMMUNE) is available for oral administration as a solution containing 100 mg/ml of vehicle (12.5% ethanol in oil); this solution is mixed with milk or orange juice immediately before administration. The intravenous formulation contains 50 mg of cyclosporine/ml of vehicle (33% ethanol in polyoxyethylated castor oil); it is diluted with 0.9% sodium chloride or 5% dextrose immediately prior to infusion. Oral treatment is initiated 4 to 24 hours prior to transplantation with a dose of 15 mg/kg; this dose (given once daily) is continued for 1 to 2 weeks postoperatively. Thereafter, the dosage is reduced each week until a maintenance dose of 3 to 10 mg/kg per day is reached. Dosage is generally guided by signs of renal toxicity, as judged from changes in creatinine clearance. Care must be taken in patients with renal transplants not to confuse rejection with the renal toxicity of cyclosporine. For this reason, biopsies of grafts are generally performed to provide definitive evidence of potential rejection. The concentration of cyclosporine in the circulation is monitored 24 hours after single oral daily doses. The commonly employed methods measure both intact cyclosporine and a number of its metabolites (Vine and Bowers, 1988; McMillan, 1989); some of these have immunosuppressive activity (Freed *et al.*, 1987). Values of 250 to 800 ng/ml in whole blood or 50 to 300 ng/ml in plasma are generally acceptable. A more accurate estimation of intact cyclosporine can be achieved by methods that employ monoclonal antibodies (Quesniaux *et al.*, 1987) or high-performance liquid chromatography (HPLC). With HPLC, concentrations of intact cyclosporine in whole blood of 100 to 150 ng/ml are considered to be in the therapeutic range.

In patients who are unable to tolerate cyclosporine orally, the diluted intravenous formulation is infused slowly over a period of 2 to 6 hours or longer. The daily dose (usually 5 to 6 mg/kg) should be only one-third the oral dose. Because frequent reactions occur to the vehicle in the intravenous formulation of cyclosporine, intravenous administration should be discontinued as soon as the patient is able to tolerate oral medication. Some patients who are to receive liver transplants have compromised renal function from the hepatorenal syndrome. Immunosuppressive treatment of such patients may be started with azathioprine and prednisone and changed to a combination of cyclosporine and prednisone after renal function has improved (usually 1 week).

CYTOTOXIC AGENTS

AZATHIOPRINE

Many cancer chemotherapeutic agents cause bone marrow toxicity and conse-

quent immunosuppression. This prompted attempts to use some of these agents for the prevention of allograft rejection. Azathioprine (combined with prednisone) has been the mainstay of attempts to suppress rejection of transplanted organs for 2 decades and has made renal transplantation an acceptable procedure (Elion and Hitchings, 1975).

In the body, nucleophiles such as glutathione cleave the prodrug azathioprine to mercaptopurine; this purine analog is subsequently converted into mercaptopurine-containing nucleotides that exert effects on the synthesis and utilization of precursors of RNA and DNA (*see* Chapter 52). Although experiments *in vitro* indicate that the immunosuppressive potency of azathioprine is greater than that of mercaptopurine, results *in vivo* are less clear (Wolberg, 1988). As compared with mercaptopurine, the apparently more favorable therapeutic effect of azathioprine may reflect differences in pharmacokinetic properties and local conversion of azathioprine to mercaptopurine at sites that are more conducive to specific effects on the immune system.

Concomitant administration of allopurinol and azathioprine engenders the hazard of overdosage with azathioprine because oxidation of mercaptopurine to inactive metabolites by xanthine oxidase is greatly reduced by allopurinol (*see* Chapter 26). Reduction of the normal dosage of azathioprine by 65 to 75% is recommended for patients who are also receiving allopurinol.

A regimen of azathioprine combined with both cyclosporine and prednisone is employed for suppression of organ rejection in some centers. Because of evidence that this regimen causes a high incidence of malignancies and infectious complications, physicians in other centers reserve this combination of drugs for patients who do not respond adequately to cyclosporine and prednisone (Salaman and Griffin, 1985). Azathioprine has also been approved for the treatment of severe refractory rheumatoid arthritis in nonpregnant adults (Hunter *et al.*, 1975).

Hematological toxicity manifested as leukopenia and thrombocytopenia must be monitored carefully to guide dosage of azathioprine. Nausea and vomiting are common, but generally do not limit treatment. Although hepatic toxicity is rare, a severe hepatic veno-occlusive disease has been seen in some patients with transplants.

Preparations and Dosage. *Azathioprine* (IMURAN) is supplied for oral administration in 50-mg tablets and in vials that contain the equivalent of 100 mg as the Na^+ salt for intravenous injection. Prophylactic therapy is usually initiated with daily doses of 3 to 10 mg/kg, 1 or 2 days prior to renal transplantation or on the day of the operation; the usual daily maintenance dose is 1 to 3 mg/kg. Treatment of rheumatoid arthritis is initiated at daily doses of 1 mg/kg, given in one or two portions. After 6 to 9 weeks, the daily dose is escalated slowly to a maximum of 2.5 mg/kg.

METHOTREXATE

In addition to its use as an antineoplastic agent (*see* Chapter 52), methotrexate is employed alone or in combination with cyclosporine for prophylaxis of graft-versus-host disease in bone marrow transplantation. It is also useful in selected forms of autoimmune and inflammatory disease. Methotrexate is a potent inhibitor of dihydrofolate reductase, with consequent effects on folate-requiring reactions in the biosynthesis of thymidylate and purines. The immunosuppressive activity of methotrexate presumably reflects inhibition of the replication and function of T cells and possibly B cells because of a relatively selective action on DNA synthesis. In leukemic patients who receive bone marrow transplants, there is some evidence that recurrence of disease is reduced in those given methotrexate as compared with those treated with cyclosporine, presumably because of the intrinsic antileukemic effect of methotrexate (*see* International Bone Marrow Transplant Registry, 1989).

Methotrexate has recently been approved for the treatment of severe, active rheumatoid arthritis in adults (Tugwell *et al.*, 1987; Weinblatt and Kremer, 1988) and of psoriasis that is refractory to other therapy (Roenigk *et al.*, 1988). For rheumatoid arthritis, the usual dose of methotrexate is 7.5 mg given once a week; this amount can be taken in three divided portions at 12-hour intervals. The dose may be increased slowly to a maximum of 20 mg per week. Similar dosage schedules are used to treat psoriasis (*see* Chapters 52 and 65).

The toxicities that result from the long-term administration of low doses of methotrexate are distinct from those associated with its use as an antineoplastic agent. Hepatic fibrosis and cirrhosis have been reported in as many as 30 to 40% of patients with psoriasis who were treated with the drug (Roenigk *et al.*, 1988); however, a lower incidence was found in a group of 210 patients with rheumatoid arthritis (Shergy *et al.*, 1988). In both diseases there was evidence of progressive dose-related hepatic changes during extended periods of treatment with methotrexate; these were correlated with ingestion of ethanol. Both acute and chronic nonseptic pneumonitis also occur in patients with rheumatoid arthritis. This toxic manifestation is generally reversible, but the mechanism and risk factors associated with this complication are not known. Patients with psoriasis have a much lower incidence of pulmonary toxicity.

CYCLOPHOSPHAMIDE

Details of the activation, mechanism of action, and antineoplastic activity of cyclophosphamide are presented in Chapter 52. Cyclophosphamide is activated by a cytochrome P_{450}–catalyzed reaction in the liver and other tissues to form alkylating species that interact with DNA. Cyclophosphamide is the primary agent used to ablate lymphoid elements in patients who are to receive bone marrow transplants. Very large single doses are employed, as compared with those used conventionally in cancer chemotherapy; toxic effects, especially chemical cystitis and cardiomyopathy, must be monitored carefully.

ANTIBODIES

Several preparations of antibodies have been approved as immunosuppressive agents. Some of these antibodies interact with lymphoid cells, leading either to blockade of their function (OKT3) or to their destruction (antithymocyte globulin). Most currently available preparations are from nonhuman sources and, hence, incur the potential for the development of anti-idiotypic antibodies, even in an immunosuppressed host. Nevertheless, their ability to lower the number and suppress the function of selected types of normal lymphoid cells has provided an important means to treat acute episodes of rejection in recipients of transplanted organs, as well as to prevent (and treat) graft-versus-host reactions in recipients of bone marrow transplants (*see* Seaman and Wofsy, 1988).

LYMPHOCYTE IMMUNE GLOBULIN

Several preparations of antithymocyte or antilymphocyte sera have been employed on an experimental basis. The preparation currently marketed (prepared in horses) can sharply lower the number of thymus-derived lymphocytes and inhibit normal responses of T cells. Since patients who receive this agent are also being treated with other immunosuppressive agents, allergic reactions to the equine protein are not as frequent or severe as would be expected. The half-life of the preparation ranges from 3 to 9 days in patients who are receiving other immunosuppressants. Chills and fever, leukopenia, thrombocytopenia, and skin reactions are seen in about 5% of patients. Anaphylaxis is a potentially serious reaction, although it occurs in only 1% of patients. The primary use of these preparations (in conjunction with other immunosuppressive agents) has been in acute graft rejection; they are also used for the treatment of severe aplastic anemia.

Preparation and Dosage. *Lymphocyte immune globulin* (antithymocyte globulin, equine; ATGAM) is available as a solution (50 mg/ml) for intravenous injection. The usual daily dose for adults is 10 to 30 mg/kg infused in saline through an in-line filter over a period of 4 or more hours. A central or other large vein is used to minimize the incidence of phlebitis.

ANTIBODIES AGAINST THE CD3 COMPLEX

Monoclonal antibodies provide a selective approach to immunosuppression; they also permit the administration of a homogeneous protein rather than a crude fraction of serum (Kung *et al.*, 1979). The primary focus has been on the receptor site for antigens on T cells and on a 20,000-dalton glycoprotein in the CD3 complex (*see* above). The commercially available preparation is a murine immunoglobulin (IgG_{2a}) termed OKT3. When complexed with its antigen (CD3), this monoclonal antibody blocks the function of all T cells that bear this receptor, presumably by preventing the initiation of signal transduction that is essential for cellular activation. When administered to patients, OKT3 causes a rapid decrease in the expression of the CD3 antigen on peripheral lymphocytes (Chatenoud *et al.*, 1982), but changes in the expression of CD3 are not apparent in lymphocytes within the graft.

Monoclonal antibody to CD3 is indicated as an adjuvant to other immunosuppressants in patients who are experiencing acute rejection of a renal allograft (Ortho Multicenter Group Transplant Study, 1985) or in prophylactic regimens (Filipovich *et al.*, 1985). When this antibody is used, the dosage of glucocorticoids and azathioprine should be reduced, and treatment with cyclosporine should probably be stopped. A large

fraction of patients experience chills, fever, and other adverse effects, including dyspnea, chest pain, wheezing, gastrointestinal disturbances, and tremor. Potentially fatal pulmonary edema occurs in about 1% of patients after the first dose, and a reversible central nervous system syndrome that is characterized by fever, headache, photophobia, and neck stiffness is common; seizures occur rarely. These signs and symptoms may be caused by the release from the affected T cells of several lymphokines that are known to elicit similar reactions. Infections occur at a rate comparable to that seen during treatment with full doses of glucocorticoids. Lymphoproliferative syndromes or lymphomas have also occurred.

Preparation and Dosage. *Muromonab-CD3* (ORTHOCLONE OKT3) is available as a solution (1 mg/ml); the solution is filtered before use and is injected intravenously as a bolus. The total daily dose is 5 mg, and treatment is continued for 10 to 14 days. In order to minimize acute reactions, the first dose should be preceded by the intravenous administration of methylprednisolone (1 mg/kg), followed by 100 mg of hydrocortisone 30 minutes later. Patients are monitored at intervals for the appearance of anti-idiotypic antibodies in the event that additional courses of therapy are necessary.

$Rh_o(D)$ IMMUNE GLOBULIN

A highly specific form of immunological therapy is employed in $Rh_o(D)$-negative mothers whose immune system is exposed to $Rh_o(D)$-positive blood as a result of fetomaternal hemorrhage during abortions, amniocentesis, abdominal trauma, or even full-term deliveries. Administration of concentrated human antibodies against this erythrocyte antigen to the mother blocks the immune response, thus eliminating the risk of hemolytic disease in infants during subsequent pregnancies (Bowman, 1985; Thornton *et al.*, 1989). Large amounts of $Rh_o(D)$ immune globulin can also be given to patients after accidental transfusion with mismatched blood. Several preparations of concentrated human IgG antibodies directed against the $Rh_o(D)$ antigen on red cells are available for intramuscular administration at the time of a prenatal incident or prophylactically postpartum. These preparations should *not* be used in the infant, in mothers who have been sensitized previously, or in $Rh_o(D)$-positive patients. Minor reactions at the site of injection and mild fever have been observed.

Preparations and Dosage. *$Rh_o(D)$ immune globulin* (RHOGAM, GAMULIN RH, others) is available for intramuscular injection in vials or syringes, each of which is capable of neutralizing approximately 15 ml of packed $Rh_o(D)$-positive erythrocytes. Larger doses are used in proportion to the amount of mismatched blood transfused or the estimated degree of fetal–maternal hemorrhage. $Rh_o(D)$ immune globulin is best administered within 72 hours of delivery; postpartum treatment may be omitted if delivery occurs within 3 weeks of the last dose, unless the fetal–maternal hemorrhage is greater than the equivalent of 15 ml of packed erythrocytes.

$Rh_o(D)$ immune globulin is also available in reduced dosage preparations (MICRHOGAM, MINI-GAMULIN RH, others). Each vial or syringe is capable of neutralizing 2.5 ml of packed $Rh_o(D)$-positive red cells. This preparation is used prophylactically in $Rh_o(D)$-negative women after termination of pregnancies up to and including 12 weeks' gestation unless either the father or fetus is $Rh_o(D)$ negative. The full-strength preparation is employed at or beyond 13 weeks of gestation.

OTHER IMMUNOSUPPRESSIVE AGENTS

Adrenocorticosteroids. The lympholytic and antiinflammatory actions of adrenocorticosteroids are frequently used to advantage in immunosuppressive regimens (*see* Chapter 60). As a prophylactic measure to prevent rejection, prednisone is given for about 4 days in doses of 0.5 to 2.0 mg/kg; the dose is then tapered for maintenance to 5 to 10 mg per day. These doses can be reduced somewhat when cyclosporine is administered concomitantly. During episodes of acute organ rejection, 500 to 1500 mg of methylprednisolone is given intravenously for several days; lower doses are used for acute graft-versus-host disease after bone marrow transplantation. Glucocorticoids are commonly given intravenously before and after administration of lymphocyte immune globulin or monoclonal antibodies to minimize the incidence of reactions to these preparations. Because of the high dosage of glucocorticoids required for immunosuppression, major side effects are common; these include cushingoid reactions, psychoses, glucose intolerance, infections, hypertension, cataracts, skin fragility, bone dissolution, and impaired growth in children.

Sulfasalazine. Sulfasalazine was originally designed as a combination of an antimicrobial sulfonamide (sulfapyridine) and an antiinflammatory salicylate (5-aminosalicylic acid; mesalamine) for the treatment of ulcerative colitis and other inflammatory bowel diseases. Its usefulness in the treatment of rheumatoid arthritis has been investigated more recently (Pinals, 1988; Pullar, 1989). Although the therapeutic effects of sulfasalazine in ulcerative colitis have been attributed to the liberation of mesalamine in the colon, it would appear that sulfapyridine is responsible for its beneficial effects in rheumatoid arthritis. The mechanism of its therapeutic action has not been established, but there is evidence for suppression of the activity of natural killer cells (Gibson and Jewell, 1985) and impairment of lymphocyte transformation (Sheldon *et al.*, 1987).

FK-506. FK-506 is a newly described macrocyclic lactone–lactam antibiotic with immunosuppressive properties that are similar to those of cyclosporine (Starzl *et al.*, 1987; Tocci *et al.*, 1989; Todo *et al.*, 1989). It is between 50 and 100 times more potent than cyclosporine *in vitro*. FK-506

binds to a small protein with peptidyl proline isomerase activity that closely resembles cyclophilin; however, the specificity of binding is very different (Harding *et al.*, 1989; Siekierka *et al.*, 1989). Preliminary results from clinical studies are very encouraging and suggest that its therapeutic usefulness may be similar to that of cyclosporine, but with less toxicity. It must be appreciated, however, that the spectrum of problems engendered by immunosuppression with FK-506 is likely to be similar to that with cyclosporine. FK-506 has caused serious vasculitis and renal damage in some animal species.

Methoxsalen. A novel treatment method termed *extracorporeal photophoresis* causes beneficial effects in the majority of patients with the erythrodermic form of cutaneous T-cell lymphoma when disease is resistant to conventional therapy (Edelson *et al.*, 1987). Methoxsalen is given orally 2 hours before the removal of whole blood, which is then irradiated with ultraviolet light after it has been diluted in an appropriate medium. The irradiated cells, including the target T cells, are returned to the patient, and the process is repeated the next day. These 2-day courses are repeated at 4- to 8-week intervals. It has been postulated that photosensitization of the malignant T cells occurs and that this facilitates a further immune reaction against the neoplastic population of cells in the body. This approach is being extended to autoimmune and related diseases based on preliminary results in experimental systems (Perez *et al.*, 1989).

Thalidomide. Thalidomide is being investigated as an immunosuppressant for use in bone marrow transplantation. Despite its teratogenic properties, this sedative was shown to have immunosuppressive properties in experimental systems, as well as antiinflammatory activity in lepromatous leprosy (Barnhill and McDougall, 1982). Clinical trials are in progress (Vogelsang *et al.*, 1988).

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